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Pilosocereus arrabidae (Byles & Rowley) of the Grumari sandbank, RJ, Brazil: Physical, chemical characterizations and antioxidant activities correlated to detection of flavonoids

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ABSTRACT

The fruits of *Pilosocereus arrabidae* (Cactaceae) is found in the Grumari shoal, located in the Rio de Janeiro state, Brazil and, several studies have already highlighted the importance of consuming its fruits. This work aims to investigate the physical, mineral and physicochemical properties of its fruits as well as to establish the knowledge about their chemical constituents and antioxidant properties. The peel (Pe) and pulp (Pu) extracts were obtained by maceration with the following solvents: hexane (HX), dichloromethane (DCL), ethyl acetate (EAC) and ethanol 70% (ET). The extracts were analyzed by Gas Chromatography coupled to Mass Spectrometry (GC–MS) and, by High Performance Liquid Chromatography (HPLC–DAD and HPLC–MS) for its chemical investigation. For the antioxidant activity investigations the ORAC, DPPH and ferrous ion chelating capacity (FICC) tests were performed. As results, we found higher yields for peels (72%) compared to pulps (28%). By the physical–chemical analyses we point out the fruits as a good source of fiber (pulp: 6.01 mg/100 g; peel: 8.02 mg/100 g). The minerals were analyzed by the method of issuing flames and indicated high levels of selenium (DRI for pulp and peel: 147%) and manganese (DRI pulp: 97.69% and DRI peel: 269.56%). The total flavonoid contents of the fruits performed by HPLC–DAD presented 0.45 μg equiv. in quercetin/mL of peel EAC extract and 0.25 μg equiv. in rutin/mL of pulp EAC extract. The antioxidant activities by the ORAC, FICC and DPPH methods indicated that the ET extracts showed antioxidant activities above the standards adopted for the tests. Among these, we highlight the ET extract of the pulp with EC_{50} of 17.57 ± 0.27 μg/mL, lower than *Ginkgo biloba* EGB761® (23.40 ± 0.04 mg/mL). By the FICC test the EAC extract of the peel and pulp showed 70.0% and 53.4% activity, respectively, at 500 mg/mL, higher than the standard quercetin (50.0%). By the HPLC–DAD and HPLC–MS methods there were detected, for the first time on this species, the presence of the following flavonoids on the EAC extracts: quercetin, rutin, catechin, dihydrokaempferol, quercetin 3 or 4′-O-glucoside, kaempferol and isorhamnetin. By the GC–MS analysis there were detected on the DCL extracts saturated fatty acids (palmitic and stearic) and, on the HX extracts, methylated sugars (peel) and menthol (pulp). To sum up, the fruits of *P. arrabidae* display antioxidant potential correlated to flavonoid presence, and, high levels of selenium, manganese and fibers, characteristics that can promote beneficial effects on human health.

1. Introduction

The species *Pilosocereus arrabidae* (Byles & Rowley) is a fruitful exotic vegetal species belonging to the family Cactaceae that comprises from 120 to 200 genera including from 1500 to 2000 species. This species is found in the Grumari sandbank (Rio de Janeiro State), which belongs to the Tropical Rain Forest Submontane. The *P. arrabidae* has a columnar form of life, fruits with seeds and, symmetric black staining that is eaten by birds, lizards and bats (Zappi, 1994). The *Pilosocereus* differs from other genera of Cactaceae because it has a globular-flattened fruit, dehiscent by irregular fissures and funicular pulp. Its branches are relatively short, straight and columnar and, its flowers open at night when they are pollinated by bats (Taylor & Zappi, 2004). It is the third most

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representative genus of the family in Rio de Janeiro, including three species: *P. ulei* (Byles & Rowley), *P. brasiliensis* (Britton & Rose) and *P. arrabidae* (Byles & Rowley) (Calvente, Freitas, & Andreata, 2005). The cactaceous family is already recognized by the presence of flavonoids such as rutin and quercetin (Alimi, Hfaiedh, Bouoni, Sakly, & Ben Rhouma, 2011; Semedo, 2012) and, by their high fiber content. As an example we mention the *Pitaya* fruits, that present a composition rich in fiber, magnesium, potassium, and flavonoids. In addition, these fruits have already been described by their rich nutritional composition and, by a significant antioxidant activity detected by the DPPH method (Ginestra et al., 2009; Stintzing & Carle, 2005; Tenore, Novellino, & Basile, 2012). On this context, it is important to highlight that the food and pharmaceutical industries have been investing on the discovery of new natural products with antioxidant potential. So, it is therefore of paramount importance to invest on the chemical analysis of *P. arrabidae* fruits, as well on its nutritional composition. No studies have been previously published concerning the physical, mineral and chemical constitution of its fruits as well as none based on its antioxidant activities and flavonoid contents, arousing interest for this present study.

2. Material and methods

2.1. Raw material

The fruits of *P. arrabidae* were collected in a typical area of native vegetation from the Grumari sandbank (south latitude 23°02′ and west longitude 43°31′). The fruits were ripe and presented uniform characteristics regarding the size and color. The fruits were identified by Prof. Dra. Alice Sato from the Federal University of the Rio de Janeiro State — UNIRIO. The voucher specimen was deposited under the number HUNI661 at the herbarium of the Federal University of the Rio de Janeiro State — UNIRIO. The fruits (n = 10 total fruits) were kept in a freezer and then manually separated into peel (427.2 g) (Pe) and pulp (395.4 g) (Pu) and subjected to maceration at room temperature for 5 to 7 days with the following solvents with different polarities: *n*-hexanes (4 × 300 mL) (HX), dichloromethane (4 × 300 mL) (DCL), ethyl acetate (4 × 300 mL) (EAC) and 70% ethanol (4 × 300 mL) (ET). The extractive solutions were concentrated in a rotary evaporator under vacuum (Fisatom®) and then weighted for yield calculation.

2.2. Physical characterization

Individual measurements of mass, transverse and longitudinal diameters were carried out on 30 fruits by using a digital caliper rule (Mitutoyo®). The masses of the fruits (MF), pulp (MPu) and peel (MPe) were obtained by direct individual weighing on a semi-analytical balance (Gehaka, model BG 2000). The pulp and peel yield was calculated by using the formula (MPu + MPe / MF) × 100.

2.3. Chemical composition and physicochemical properties

The chemical composition and the physicochemical properties of the pulp and peel from *P. arrabidae* were assessed by using the standard methods of the Association of Official Analytical Chemists (AOAC, 2005), as follows: water content, carbohydrate, ashes, lipids, protein, dietary fiber, pH, titrable acidity and soluble solids. All analyses were performed in triplicate.

2.4. Mineral analysis

The following minerals contents were analyzed considering the pulp and peel of *P. arrabidae* fruits: sodium, magnesium, potassium, phosphorus, calcium, manganese, copper, iron, selenium and zinc. The digestions of the samples were made by wet ashing and the quantification by atomic spectroscopy and mass spectrometry (ICP-MS, Spectro Analytical Instruments GmbH®, Germany) according to the AOAC methods (AOAC, 2005). The results were expressed in mg/100 g. All analyses were performed in triplicate.

2.5. Antioxidant activity assays

2.5.1. 2,2-Diphenyl-1-picryl-hydrazil (DPPH) method

The antioxidant activity was determined by the DPPH method with the following samples: PuET (pulp hydroalcoholic extract); PeET (peel hydroalcoholic extract); PuDCL (pulp dichloromethane extract); PeDCL (peel dichloromethane extract); PuEAC (pulp ethyl acetate extract) and PeACT (peel ethyl acetate extract). In accordance with the methodology of Ruela et al. (2011), stock solutions (1 mg/mL) of the extracts were prepared using methanol as solvent. The antioxidant activity was measured by adding 1 mL of 0.3 mM DPPH solution (Sigma-Aldrich®) into 2.5 mL of each sample solution (achieving final concentrations at 5, 10, 25, 50, 125, 250 and 500 μg/mL). For the blank, it was added to the extracts solutions 1 mL of methanol instead of DPPH solution. The negative control consisted of 1 mL of DPPH and 2.5 mL of methanol. The reactions were performed at room temperature for 30 min and then, the absorbance readings were made at 518 nm by using a spectrophotometer (Shimadzu®). The values of absorbance were converted into percentage of antioxidant activity (AOA%) and the results were expressed as EC_{50} values. The standardized extract of *Ginkgo biloba* Egb 761® was used as positive control, having been tested under the same conditions described above. All analyses were performed in triplicate.

2.5.2. Oxygen Radical Absorbance Capacity (ORAC) method

The evaluation of the antioxidant activity was also determined by the ORAC method according to Stockham, Paimin, Orbell, Adorno, & Buddhadasa (2011). For this test there were tested the same samples as described in Section 2.5.1. The assay is based on the reaction of the xanthene fluorescein with peroxyl free radicals generated by the oxidation of 2,2′-azobis-2-methyl-propanamide dihydrochloride (AAPH) with the atmospheric oxygen. The radical inactivates the fluorescein, decreasing its fluorescence. The antioxidant activity of the substance to be tested was determined by the ability to inhibit the oxidation of the fluorescein by measuring over the time the amount of fluorescence emitted. The samples were prepared in PBS at the following concentrations: 2.5, 5.0, 7.5, 10, 12.5, 15, 17.5 and 20 mg/mL. Each sample (20 μL) was added to 120 μL of fluorescein (Isofar®) and the solutions were incubated at 37 °C for 10 min in 96-well microplates (SPL — Labiotec). Then, 60 μL of AAPH was added (Aldrich®) and again incubated at 37 °C for 1 h and 35 min. The absorbance was measured by a Fluostar Optima fluorimeter (BMG Labtech®). To check the interference of the sample absorption, a negative control was prepared with PBS (20 μL), 120 μL of fluorescein and 60 μL of AAPH. A blank containing PBS (80 μL) and 120 μL of fluorescein was also prepared. A solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich®) was used as standard of high antioxidant capacity and tested under the same conditions and concentrations described above. All tests were performed in triplicate.

2.5.3. Ferrous Ion Chelating Assessment Capacity (FeIQC) method

The iron chelating ability was determined according to the method of Wang, Jónsdóttir, & Ólafsdóttir (2009) with minor modifications. In this assay there were evaluated the same samples as described in Section 2.5.1. The samples were prepared in ethanol at the following concentrations: 0.1, 1, 10, 100 and 1000 mg/mL. A volume of 100 μL of each sample was added to 140 μL aqueous solution of 2 mM $FeCl_2$ (Merck®). The reaction was initiated by addition of 10 μL of 5 mM ferrozine (Sigma-Aldrich®). The solutions were maintained under stirring for 10 min at room temperature. After incubation, the absorbance was measured in a microplate reader at 562 nm (Biot-Tek Instruments Inc.). To check the interference of the sample absorption, blanks were

prepared with distilled water (10 μL) in place of the solution of ferrozine and, 100 μL distilled water in place of the sample solution. The EDTA–Na_2 (0.1; 1; 10; 100 and 1000 mg/mL) (Merck®) and the standard quercetin (0.001, 0.01, 0.1, 1 and 10 mg/mL) (Sigma-Aldrich®) were used as positive controls under the same conditions. The chelating ability of ferrous ions (CQFe) was calculated as a percentage. All tests were performed in triplicate.

2.6. Chromatographic analysis

2.6.1. Gas Chromatography Coupled to Mass Spectrometry (GC–MS) analysis for chemical characterization of the n-hexanes and dichloromethane extracts constituents

The analyses were performed on a Shimadzu® GCMS-2010 appliance with a QP2010 interface and electron impact. The column used for the analysis was Rtx-5MS (L = 30 m, d = 0.25 mm) and the carrier gas: helium. The temperature of the interface was 300 °C and, the injector was 270 °C. The gradient elution protocol consisted of a column temperature that varied from 60 °C (1 min) to 290 °C (10 °C/min), maintained at the end for an additional 16 min. For the derivatization procedure, the *n*-hexane and dichloromethane extracts (2 mg) of the pulp (Pu) and peel (Pe) were dissolved into 200 μL of the reagent (N-Methyl-N-trimethylsilyl-trifluoroacetamide), subsequently homogenized and then left at room temperature for 30 min. At the end of the reaction 2 mL of ethyl acetate was added and the mixtures were stirred vigorously. From the solutions, 1 μL was injected into the GC–MS by using an automatic injector.

2.6.2. High Performance Liquid Chromatography coupled to Diode-array Detector (HPLC–DAD) analysis for chemical identification and quantification of the total flavonoids

The ethyl acetate extracts of the pulp and peel were evaluated by HPLC–DAD (Shimadzu 2010A) in an automatic apparatus consisting of two LC–10AD pumps, DGU-12A degasser, SIL-10AD injector and column oven CTO-10A. Samples (10 mg) were diluted in 2 mL (acetonitrile) and 10 μL of each sample was injected. The gradient elution protocol consisted of different ratios of the solvents A and B (A: water + 1% H_3PO_4, B: acetonitrile) in a total of 45 min with a flow rate of 1 mL/min as follows: 25% B (0–10 min); 25–50% B (10–20 min); 50–70% B (20–25 min); 70–95% B (25–35 min); 95% B (35–40 min); 95%–100% (40–43 min) and 100% B (43–45 min). The column used was a Chromasil: C18 — 100 × 3 × 2.6 mm. In order to quantify the main flavonoids present in the ethyl acetate extracts (PuEAC; PeEAC) of the fruits of *P. arrabidae* a calibration curve with authentic commercialization quercetin and rutin standards (SIGMA-ALDRICH®) was adopted in different concentrations, namely, 0.01; 0.025, 0.05, 0.1; 0.5; 1 and 2.5 mg/mL at the same conditions described above.

2.6.3. High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC–MS) analysis for chemical identification of the flavonoids

The HPLC–MS analysis of the PeEAC and PuEAC extracts were performed on a Shimadzu® UFLC Modelo Nexera. The separation by HPLC was carried out with a mobile phase composed of acetonitrile and water with phosphoric acid (pH 1.8) at a flow-rate of 1 mL/min (gradient). The volume of the sample injected was 10 μL at a concentration of 5 mg/mL on a column Kinetex: C18 — 100 × 3 × 2.6 mm at a temperature of 30 °C. The gradient elution protocol consisted of 70%B (0–10 min); 70–95%B (10–20 min) and 95%–100%B (20–25 min). The identification of rutin and quercetin on the samples was carried out by comparing their retention times with standards and, by detection of adduct molecular ions. The other flavonoids were identified by the molecular ions and adduct molecular ion detection.

3. Statistical analysis

The results represent the mean ± standard deviation of three independent experiments. The comparison of the data was evaluated beyond the software Graph pad Prism Program, in which there were evaluated the variance analyses by ONE-WAY ANOVA followed by the Tukey and Student T tests considering it statistically significant when $p < 0.05$.

4. Results and discussion

4.1. Physical characterization

The physical characteristics of the *P. arrabidae* fruits are described in Table 1. As can be seen, the longitudinal, transverse and average individual weight data measured were 7.0 cm, 4.1 cm diameter and 32 g, respectively. The yield of the pulps established for the fruits of *P. arrabidae* was 28%. In the study developed by Adams (2007) the pulp from the fruit of *Pilosocereus gounellei* presented a yield of 18.22%, lower than that measured for *P. arrabidae*. It is important to highlight that this physical characteristic for the fresh fruits is of great importance for the food market since a larger amount of pulp favors its processing for selling frozen (Rufino et al., 2010).

4.2. Chemical composition

As can be seen in Table 2, the titratable acidity attributed to the pulp and peel of *P. arrabidae* was 0.27 mg and, 0.35 mg of citric acid per 100 g^{-1}, respectively, qualifying them as a source of this component. About the pH, it was recorded for the pulp and peel the following values: 4.69 and 4.49, and, in terms of soluble solids, it was registered at 10° and 12° Brix, respectively. These data allow us to assert that the fruits present levels of soluble solids suitable for industrial processing (Trevisan, Gonçalves, Gonçalves, Antunes, & Herter, 2008).

The lipid levels registered were 0.87 and 0.48 g/100 g for pulp and peel, respectively. Le Bellec (2003) featuring the pitaya pulp lipid contents reported levels in the range of 1.17 g/100 g, values higher than those found in this work. This fact can be related to differences according to genetic factors, growing conditions and degree of maturity of the species (Trevisan et al., 2008). The values of total fibers found for the *P. arrabidae* fruits (pulp: 6 g/100 g; peel: 8 g/100 g) is considered relevant by the Brazilian regulation, which determines a minimum of 6 g/100 g. Similar values were found for fruits largely consumed by the population as guava (6 g/100 g) and pear (4 g/100 g) (Giuntini, Lajolo, & Menezes, 2003). In contrast, due to the low content of macronutrients, the *P. arrabidae* pulp and peel presented a low total energy value.

The ground fruits of *P. arrabidae* showed water contents for the pulp (90%) and peel (89%) slightly lower compared to the cactaceous pitaya (91% to 95%). It is important to highlight that water content is the major component of fruits and vegetables, being present in a total of 80 to 95%

Table 1
Physical characteristics of the *Pilosocereus arrabidae* fruits.

Physical characteristics	Mean[a] ± SD[b]	Amplitude	
		Minimum	Maximum
Longitudinal diameter (cm)	7.0 ± 0.76	3.5	9.8
Transverse diameter (cm)	4.1 ± 2.20	3.3	4.8
Height (cm)	3.6 ± 2.30	2.3	4.8
Mass (g)			
Fruit	32.0 ± 8.02	24	38
Peel	21.6 ± 0.15	18	29
Pulp	8.9 ± 0.03	5.6	12
Pulp yield (%)	28.0 ± 0.05	18	36

[a] Mean of 30 fruits.
[b] Standard deviation.

Table 2
Chemical characteristics of *Pilosocereus arrabidae* fruits: pulp and peel.[A].

Chemical characteristics	*Pilosocereus arrabidae* fruits Mean[A] ± SD[B]	
	Pulp	Peel
Titrable acidity (%)	0.27 ± 0.00[a]	0.35 ± 0.00[b]
Soluble solids (°)	10.00 ± 0.35[c]	11.00 ± 0.77[c]
pH	4.69 ± 0.03[d]	4.49 ± 0.02[d]
Lipids (g 100 g^{-1})	0.87 ± 0.05[e]	0.48 ± 0.17[f]
Protein (g 100 g^{-1})	2.69 ± 0.03[g]	1.91 ± 0.25[h]
Ash (g 100 g^{-1})	0.04 ± 0.00[i]	0.29 ± 0.45[j]
Carbohydrate (g 100 g^{-1})	0.40 ± 0.08[l]	5.70 ± 0.45[m]
Total energy value (kcal 100 g^{-1})	20.20 ± 0.05[n]	35.34 ± 0.24[o]
Total dietary fiber (g 100 g^{-1})	6.01 ± 0.10[p]	8.02 ± 0.70[q]
Water content (%)	90.01 ± 0.00[r]	89.01 ± 0.06[r]

Means followed by the same letter at each line do not differ statistically by Student T test at 5% ($p < 0.05$).
[A] Means of experiments performed in triplicate.
[B] Standard deviations.

in relation to the total composition (Kays, 1997). According to Le Bellec (2003), Stintzing, Schieber, & Carle (2003) and Vaillant, Perez, Davila, Dornier, and Reynes (2005), the cactus moisture contents are registered beyond 75–88%.

4.3. Mineral contents

As can be seen in Table 3, the fruits of *P. arrabidae* showed elevated levels of selenium and manganese. The manganese acts in the dismutation of hydrogen peroxide, a cofactor of superoxide dismutase (Fillipin, Vercelino, Marroni, & Xavier, 2008). In addition, it acts on the incorporation of calcium into bones. The açai berry is well recognized to comprise a high concentration of manganese (6.16 mg/100 g) (Brazilian table of food composition, 2011) and, in our studies we found that the fruits of *P. arrabidae* showed a similar value for the pulp (6 mg/100 g) and a smaller value for the peel (2 mg/100 g). Ferreira, Gomes, Bellato, & Jordão (2002) assessed the selenium content of various fruits commonly consumed in Brazil, and, the mango was the one appointed as presenting the highest content (0.009 mg/100 g). The fruits of *P. arrabidae* showed higher values (0.05 mg/100 g) compared to that, highlighting this fruit as an important source of this mineral. It is relevant to mention that, usually, vegetal species do not present high levels of selenium, which make these data even more interesting. The selenium is a mineral associated with the protection of damages caused by oxidative stress, and, it is proposed that its intake reduce the risk of chronic diseases resulting from oxidative and inflammatory states. The scientific literature has shown that various types of dietary antioxidants, including selenium, may be effective in suppressing the activation of pro-inflammatory pathways through chelating free radical molecules (Walston et al., 2006).

Table 3
Mineral contents of the pulp and peel from *Pilosocereus arrabidae* fruits in mg per 100 g and, the DRI percentage contribution.

Variables	*Pilosocereus arrabidae* Mean[A] ± SD[B]	
	Pulp	Peel
Na (mg/100 g)	37.10 ± 0.10[a]	41.70 ± 0.07[b]
% DRI	2.47	2.78
K (mg/100 g)	305.10 ± 1.30[c]	435.12 ± 4.47[d]
% DRI	6.49	9.25
Mg (mg/100 g)	28.50 ± 0.10[e]	64.0 ± 0.80[f]
% DRI	10.96	24.65
Ca (mg/100 g)	8.70 ± 0.20[g]	47.90 ± 0.30[h]
% DRI	0.87	4.79
P (mg/100 g)	24.20 ± 0.10[i]	30.01 ± 0.10[j]
% DRI	3.45	4.28
Fe (mg/100 g)	0.41 ± 0.01[l]	0.43 ± 0.00[l]
% DRI	2.93	3.07
Zn (mg/100 g)	0.34 ± 0.00[m]	0.60 ± 0.00[n]
% DRI	4.86	8.57
Se (mg/100 g)	0.05 ± 0.001[o]	0.05 ± 0.00[o]
% DRI	147	147
Co (mg/100 g)	0.05 ± 0.002[p]	0.08 ± 0.00[p]
% DRI	5.55	8.88
Mn (mg/100 g)	2.24 ± 0.02[q]	6.20 ± 0.10[r]
% DRI	97	269

Means followed by the same letter in each line do not differ statistically by the Student T test at 5% ($p < 0.05$); DRI = Dietary Reference Intakes.
[A] Means of experiments performed in triplicate.
[B] Standard deviation.

Table 4
Antioxidant evaluations by the DPPH, ORAC and Ferrous Ion Chelating Capacity methods for the peel extracts.

Extracts	ORAC μmoles Trolox·g^{-1} Mean[A] ± SD[B]	DPPH CE_{50} (μg/mL) Mean[A] ± SD[B]	FICC % at 500 μg/mL Mean[A] ± SD[B]
Ethanol 70%	620 ± 0.18[a]	31.30 ± 0.03[a]	53.2 ± 0.22[a]
Dichloromethane	560 ± 1.22[b]	51.80 ± 0.08[b]	52.2 ± 1.12[b]
Ethyl acetate	534 ± 2.35[c]	60.76 ± 0.12[c]	70.0 ± 1.33[c]
Ginkgo biloba®	NT	23.40 ± 0.04[d]	NT
Quercetin	NT	NT	52.34 ± 1.22[d]

Means followed by the same letter in each column do not differ statistically by the Tukey test at 5% probability ($p < 0.05$); NT = not tested.
[A] Means of experiments performed in triplicate.
[B] Standard deviation.

4.4. Antioxidant activity assays

As can be seen in Tables 4 and 5, concerning the antioxidant activity evaluations, both hydroalcoholic extracts of the fruits (PuET and PeET) presented excellent antioxidant activities by the all three methods (ORAC, DPPH and AQH) compared to the other extracts (dichloromethane and ethyl acetate).

By the ORAC method the PeET and PuET exhibited the best profiles (620 and 630 μmol Trolox·g^{-1}, respectively) among the other extracts despite DCL and EAC have also presented high antioxidant levels (560 (Pe); 590 (Pu) and, 534 (Pe); 560 (Pu) μmoles Trolox·g^{-1}, respectively). In the study developed by Gonçalves (2008) with the pulp of an exotic fruit named as Camu-camu showed a high antioxidant activity by the ORAC method (790 μmol Trolox·g^{-1}). This data is closer to those established in our work for the pulp extracts of *P. arrabidae* that ranged from 560 a 630 μmol Trolox·g^{-1}.

By the DPPH method, it was also established for the hydroalcoholic extracts the highest antioxidant activities: (PeET) (EC_{50} = 31.30 μg/mL) and (PuET) (EC_{50} = 17.57 μg/mL) when compared to *Ginkgo biloba* (23.4 μg/mL). Previous work developed by Omena et al. (2012) evaluated the antioxidant capacity of ethanol extracts from the pulp and peel of exotic fruits from Brazil by the DPPH method. Among other species, they worked on the evaluation of genipap, caja and jenipabu. By this technique they noted that the pulp ethanolic extract of caja presented the best

Table 5
Antioxidant evaluations by the DPPH, ORAC and Ferrous Ion Chelating Capacity methods for the pulp extracts.

Extracts	ORAC μmoles Trolox·g^{-1} Mean[A] ± SD[B]	DPPH CE_{50} (μg/mL) Mean[A] ± SD[B]	FICC % at 500 μg/mL Mean[A] ± SD[B]
Ethanol 70% crude	630 ± 0.91[a]	17.57 ± 0.27[a]	31.22 ± 0.87[a]
Dichloromethane	590 ± 0.34[b]	95.10 ± 0.88[b]	60.01 ± 0.58[b]
Ethyl acetate	560 ± 1.02[c]	118.50 ± 1.17[c]	53.4 ± 0.43[c]
Ginkgo biloba®	NT	23.40 ± 0.04[d]	NT
Quercetin	NT	NT	50.03 ± 1.26[d]

Means followed by the same letter in each column do not differ statistically by the Tukey test at 5% probability ($p < 0.05$); NT = not tested.
[A] Experiments performed in triplicate.
[B] Standard deviation.

Table 6
Chromatographic profile obtained by GC–MS of the hexane and dichloromethane extracts of the pulp and peel of *Pilosocereus arrabidae* fruits.

Extracts	Substances	Chemical class	RT (min)	Area (%) Mean[a] ± SD[b]
n-Hexanes				
Peel	Ribofuranose	Oligosaccharides	11.1	54.5 ± 0.40
	Arabinopiranose	Oligosaccharides	10.8	24.3 ± 5.59
	Alpha Xylofuranose	Oligosaccharides	10.0	8.21 ± 0.13
Pulp	Menthol	Monoterpene alcohols	7.5	100.0 ± 0.00
Dichloromethane				
Peel	Palmitic acid	Carboxylic acids	21.4	58.0 ± 6.63
	Stearic acid	Carboxylic acids	22.4	32.0 ± 4.63
Pulp	stearic acid	Carboxylic acids	22.6	68.4 ± 1.80
	Adipol	Carboxylic acids	21.3	32.0 ± 1.33

[a] Means of experiments performed in triplicate.
[b] Standard deviation.

antioxidant capacity (6.40%), followed by the pulp and peel ethanolic extracts of genipap (10.17 and 15.43%, respectively) at 200 μg/mL. In our studies, all extracts from the pulp and peel of *P. arrabidae* presented higher antioxidant activity at lower concentrations.

By the ferrous ion chelating test all extracts exhibited relevant activities, especially the PeEAC extract, that exhibited 70% at 500 μg/mL, higher than that presented by quercetin (51%).

The antioxidant activity of compounds can be explained by different mechanisms, such as binding to free radicals, neutralizing that, as well as by the ability to chelate transition metals. This may be one explanation for the effect exhibited by the pulp and peel extracts of the *P. arrabidae* fruits.

4.5. Chromatographic analysis

4.5.1. Gas Chromatography coupled to Mass Spectrometry (GC–MS)

The GC–MS analyses were performed with the *n*-hexane and dichloromethane extracts of the pulp and peel from the fruits of *P. arrabidae*.

4.5.1.1. GC–MS analyses of the pulp extracts. As can be seen in Table 6, it was possible to detect the monoterpene menthol as the single one constituent present on the *n*-hexane extract of the pulp. The menthol is already described in the literature by presenting important anesthetic, antispasmodic, anti-inflammatory, antiulcer and antiviral properties. In addition, it is of great importance on the pharmaceutical and, in the food and cosmetic industries (Lorenzo et al., 2002). By the GC–MS analyses it was also possible to detect on the PuDCL extract the presence of stearic acid as one of the main constituents (relative area: 68.4%).

4.5.1.2. GC–MS analyses of the peel extracts. As can be seen in Table 6, it is important to highlight that, on the *n*-hexane extract there were detected the presence of methylated sugars as the main constituents (a total relative area of 86.5%). Polysaccharides are widely distributed in the plant and animal kingdoms. In fruits, the main polysaccharide extracted from the cell wall belongs to the class of pectins that are heteropolysaccharides constituted of a linear backbone of α-D-galacturonic acid and its O-methylated derivatives. The ramifications with neutral sugars (arabinose, galactose, and other) are present in regions rich in rhamnose residues in which the degree of esterification, number and distribution of these residues depend on the source of the pectin (Axelos, Lefebvre, Qiu, & Rao, 1991; BeMiller, 1986, chap. 1). Thus, the presence of these sugars in the methylated form in the hexane extract of the peel may be directly associated with the presence of pectin in the fruit of *P. arrabidae*. Despite the total soluble fibers present in the peel of the fruits has not been performed in this work, the level of total dietary fiber found is considered high, which may explain the presence of these polysaccharides in the extract.

By the GC–MS analyses it was also possible to detect on the PeDCL extract the presence of palmitic and stearic acids as major compounds (a total relative area of 90%).

4.5.2. Qualitative analyses by HPLC–DAD

This methodology was employed in order to investigate the presence of the flavonoids rutin and quercetin on the ethyl acetate extracts of the pulp and peel of *P. arrabidae* fruits. The standard quercetin presented the retention time at 20.95 min while the glycoside rutin was eluted at 9.17 min. As can be seen in Fig. 1, the PeEAC extract presented quercetin as one of the major substances detected (relative area: 16.9%) (between other aglicones of flavonoids eluting from 19 to 24 min). On the PuEAC extract it was detected the flavonoid rutin as the major compound (relative area: 29.4%) (Fig. 2). The presences of both flavonoids on the referred extracts were then confirmed by HPLC–MS.

4.5.3. Quantitative analyses by HPLC–DAD

Based on the calibration curves of quercetin ($R^2 = 0.9832$) and rutin ($R^2 = 0.9901$) it was possible to quantify the referred flavonoids, as well as other flavonoids with equivalent UV spectra, on both extracts. The PeEAC extract showed 0.45 ± 0.11 μg/mL equiv. in quercetin, while the PuEAC showed 0.25 ± 0.23 μg/mL equiv. in rutin. The bioactive compounds rutin and quercetin have also been described on other species from the cactaceous family, such as, on the extracts obtained from the juice of *Opuntia ficus-indica* fruits (seeds and pieces of peel). The total flavonoid contents showed on the fruits of this species

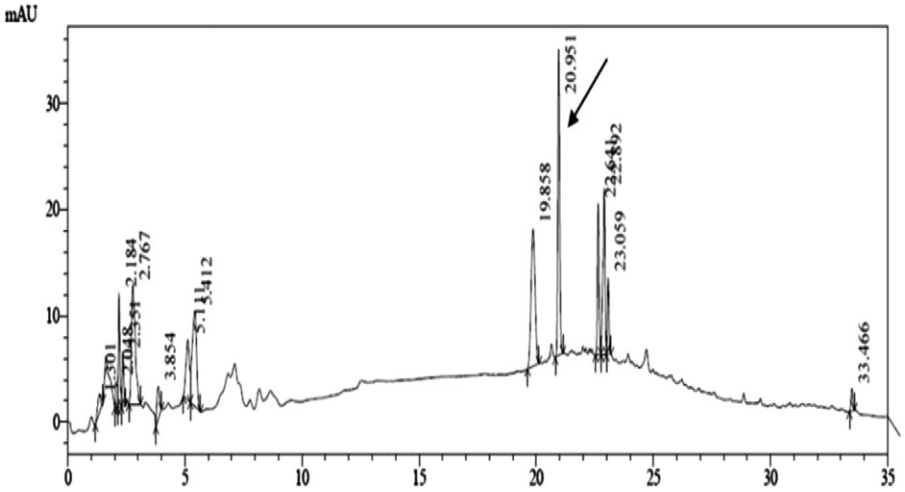

Fig. 1. Chromatographic profile of the ethyl acetate extract of the peel from the fruits of *P. arrabidae* obtained by HPLC–DAD at 355 nm. Highlight for the quercetin flavonoid (RT = 20.95 min).

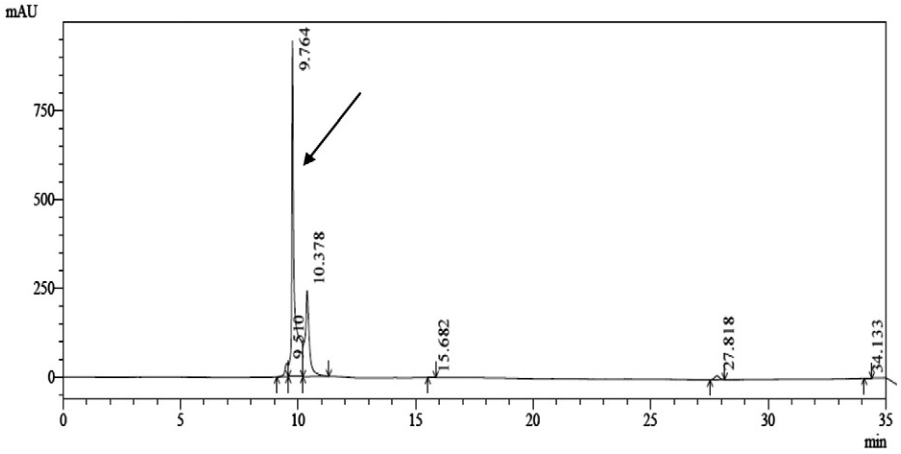

Fig. 2. Chromatographic profile of the ethyl acetate extract of the pulp from the fruits of *P. arrabidae* obtained by HPLC–DAD at 355 nm. Highlight for the rutin flavonoid (RT = 9.76 min).

were 0.5 μg/mL of rutin and, 1 μg/mL of quercetin (Semedo, 2012), closer than those established for *P. arrabidae* fruits.

4.5.4. Qualitative composition: High Performance Liquid Chromatography (HPLC) coupled to Mass-Spectrometry (MS)

In order to investigate the presence of the flavonoids on the ethyl acetate extracts of the peel and pulp of *P. arrabidae* the samples were analyzed by HPLC–ESI-MS on the negative and positive modes. The presence of quercetin and rutin on the extracts was confirmed by the qualitative evaluation of the retention times, molecular ions and adduct detections. These data were corroborated by injection of commercial standards. The presence of rutin and quercetin was confirmed by the detection of the adduct ion [M − H] m/z 609.2 and [M − H], [M + 2Na–H], [M + Cl] and [M + Na] m/z 301.1, respectively, between other, as will be discussed below. Besides these, we suggest the presence of other flavonoids as follows: catechin, isorhamnetin; dihydrokaempferol; quercetin 3 or 4′O-glycoside, kaempferol, isorhamnetin-3-O-glycoside (Fig. 3). The detection of the phenolics were based on comparative analyses of adduct ions from literature. Among these phenolic compounds, the presences of kaempferol and isorhamnetin has already been described in cladodes of *Opuntia monocantha* (Cactaceae) (Nascimento, Paixão, & Valente, 2009) and, the detection of isorhamnetin-3-O-glycoside and quercetin derivatives have already been described in fruits of *Opuntia ficus-indica* (Cactaceae) (Ginestra et al., 2009; Stintzing & Carle, 2005). The constituents identified in the respective samples are described in Table 7.

Catechin: identified on both investigated samples, peel and pulp. The presence of this phenol was possible by the detection of the adduct ions [M + H_2PO_4− 1] (m/z 387.3) and [M + K] (m/z 329.0), the last one being the most common on the spectra.

Rutin: detected in both samples analyzed. The corroboration of the rutin presence was observed by the detection of the adduct ion [M − H] (m/z 609.2).

Quercetin: observed beyond the detection of the adduct ions [M − H] (m/z 301.1), [M + 2Na–H] (m/z 347.2); [M + Cl] (m/z 337.7) and [M + Na] (m/z 325.0) on the samples, the deprotonated adduct represented by the ion m/z 301.1 being one of higher occurrences on the spectra (Fig. 4).

Quercetin 3 or 4′-O-glycoside: detected on both ethyl acetate extracts. Quercetin 3 or 4′-O-glycoside presented the following

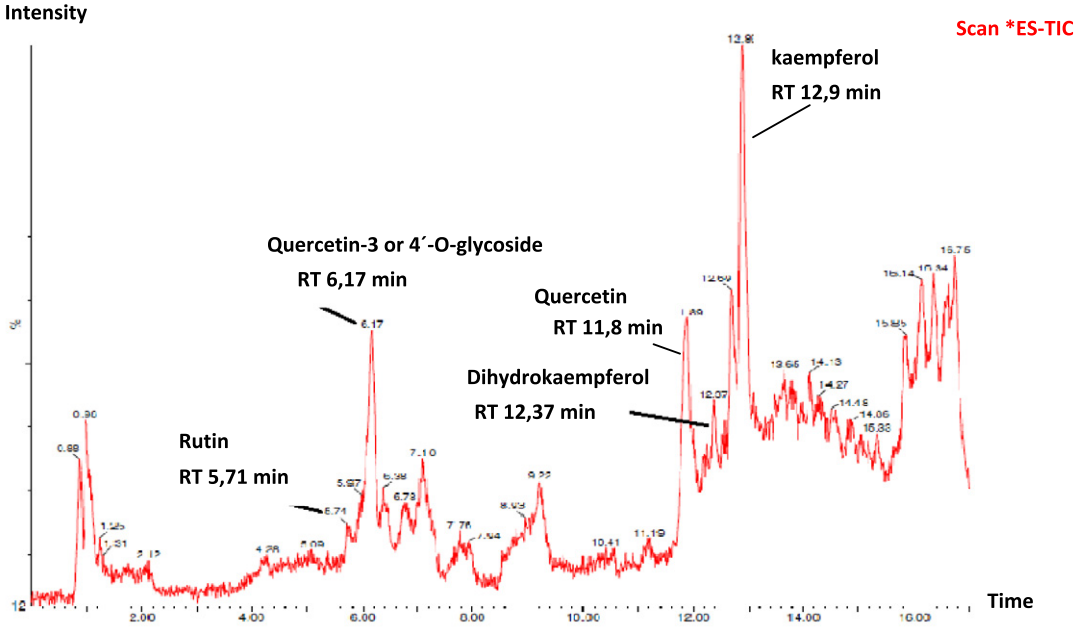

Fig. 3. Profile of total ion chromatographic (HPLC–ESI-MS) of the ethyl acetate extract of the peel of *P. arrabidae*. Highlights for the main constituents: rutin, quercetin 3 or 4′-O-glycoside, quercetin, dihydrokaempferol and kaempferol.

Table 7
HPLC–MS detection of flavonoids present on the ethyl acetate extracts of the pulp and peel of *P. arrabidae* fruits based on the molecular ion of the adducts produced by the positive and negative injection modes.

Number	Adduct ion (M +/− ion)	Molecular ion of the adduct (m/z)	Samples
1	Catechin $[290.2 + K]^+$	329.0	Pulp/peel
2	Dihydrokaempferol $[288.2 + K]^+$	327.2	Pulp/peel
3	Quercetin $[302.2 - H]^-$	301.1	Pulp/peel
4	Quercetin 3 or 4′-O-glucoside $[464.3 - H]^-$	463.2	Pulp/peel
5	Rutin $[610.5 - H]^-$	609.2	Pulp/peel
6	Kaempferol $[286.2 - H]^-$	285.1	Peel
7	Isorhamnetin $[316.2 - H]^-$	315.3	Pulp

protonated and deprotonated adduct ions: m/z 465.4 and m/z 463.2, respectively (Fig. 4).

Dihydrokaempferol: detected on both ethyl acetate extracts by the presence of the adduct ions on the spectra [M + K] (m/z 327.2), [M + Na] (m/z 311.2), $[M + PO_4 - 3]$ (m/z 383.5) and $[M + H_2PO_4 - 1]$ (m/z 385.5), being the first one detected in larger amounts (Fig. 4).

Kaempferol: detected only on the ethyl acetate extract of the peel. The kaempferol was confirmed by the detection of the adduct ions [M + K] (m/z 325.1) and [M − H] (m/z 285.1), the deprotonated adduct represented by the ion m/z 285.1 being one of the higher occurrences on the spectra (Fig. 4).

Isorhamnetin: detected only on the ethyl acetate extract of the pulp. The isorhamnetin was detected by the presence of the adduct ions [M − H] (m/z 315.3), [M + H] (m/z 317.2), $[M + H_2PO_4 - 1]$ (m/z 413.5) and [M + Cl] (m/z 351.4), being the first one detected in higher amounts on the spectra.

Flavonoids are vastly recognized by its antioxidant properties and, as could be seen, they are the major constituents present on the ethyl acetate extracts of *P. arrabidae* fruits. By this fact we assume that they are responsible for the activity displayed by the polar extracts. The PeEAC extract exhibited higher amounts of quercetin and, uniquely, the presence of kaempferol, which can be related to the higher antioxidant activity observed.

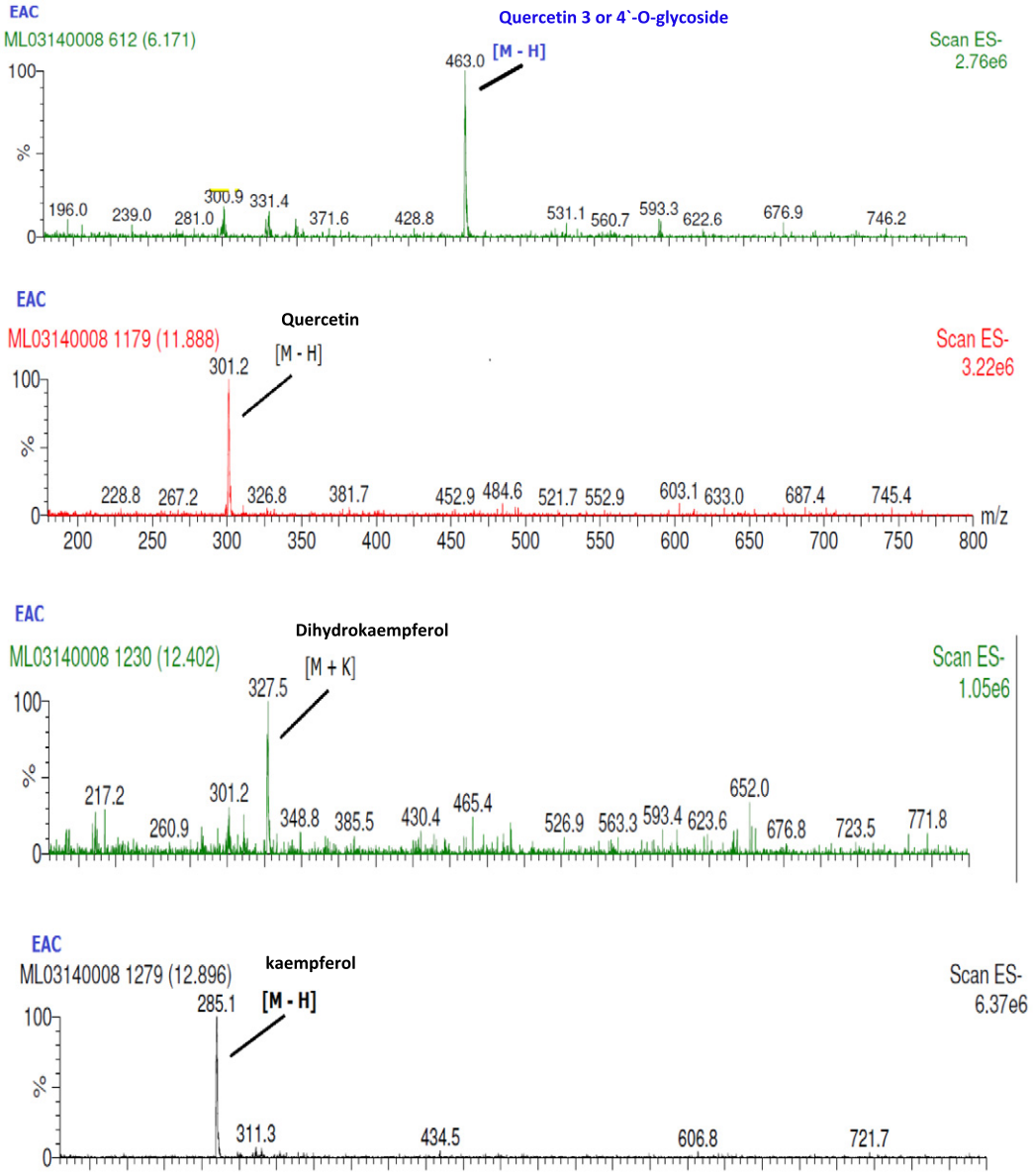

Fig. 4. Mass spectra obtained by HPLC–ESI-MS referent to the signals eluted at: 6.17 min (molecular ion from quercetin-3 or 4′-O-glycoside), at 11.88 min (molecular ion from quercetin), at 12.4 min (molecular ion from dihydrokaempferol) and at 12.8 min (molecular ion from kaempferol) detected on the ethyl acetate extract of the peels from *P. arrabidae* fruits.

5. Conclusions

The fruits of *P. arrabidae* obtained from the sandbank of Grumari, Rio de Janeiro, Brazil, showed a rounded shape, fragile shell and a good yield of pulp. The pulp and peel of *P. arrabidae* presented high humidity and low total energy intake, and, considered as a good source of selenium, manganese and total fiber. In addition, the extracts exhibited excellent antioxidant activities compared to the standards, which are related to the presence of flavonoids, detected for the first time on *P. arrabidae* extracts. The consumption of its fruits, as well as its technological research should be encouraged, due to its rich nutritional composition and bioactive compounds.

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